Affinity Purified Rabbit Anti-Phospho-5-Lipoxygenase (S523) Certificate of Analysis

ORDERING INFORMATION

Catalog Number: PPS001

Lot Numbers: 1671247

Size: 100 µL (sufficient for 10 mini-blots)

Storage: ≤ -20° C

Specificity: Human and rat ~80 kDa 5-LO phosphorylated at S523

Immunogen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-S523 of human 5-Lipoxygenase (5-LO)

Ig Type: rabbit IgG

Applications: Western Blot



Western blot of rat cortex lysate showing specific immunolabeling of the ~80 kDa doublet of 5-LO phosphorylated at S523 (Control). The phosphospecificity of this labeling is demonstrated by treatment with 1200 U of λ Phosphatase (λ -PPase) for 30 minutes before being exposed to the Anti-Phospho-5-Lipoxygenase (S523). The immunolabeling is completely eliminated by treatment with λ -PPase.

Description

5-Lipoxygenase (5-LO) is an 80 kDa, 676 amino acid (aa) nonheme iron dioxygenase. It converts nuclear membrane arachidonic acid into leukotriene A4 (LTA₄) that is then modified to produce either LTB₄ or LTC₄. 5-LO is found in leukocytes and generally exists in the cytoplasm. Upon activation, 5-LO will either remain cytosolic or undergo translocation into the nucleus. In the nucleus, its enzyme products impact gene expression; in the cytosol, its products are secreted and contribute to inflammation. 5-LO has three nuclear localization signal (NLS) motifs starting at amino acids 112, 158, and 518. Upon cell activation, Ser at 523 is phosphorylated by Protein Kinase A (PKA). This amino acid lies within the principal NLS of 5-LO. When phosphorylated, the NLS is inactivated, blocking 5-LO entry into the nucleus. This appears to impact overall leukotriene biosynthesis, and potentially serves as a regulatory mechanism for inflammation.

Preparation

Prepared from rabbit serum by affinity purification via sequential chromatography on phospho- and dephosphopeptide affinity columns.

Formulation

100 μL in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 μg/mL BSA and 50% glycerol.

Storage

For long-term storage, \leq -20° C is recommended. Product is stable at \leq -20° C for at least 1 year.

Specificity

The antibody is specific for the ~80 kDa 5-LO phosphorylated at S523 in Western blots of rat hippocampal lysates. The antibody recognizes human and rat forms of 5-LO but has not been tested in other species.

Applications

Western blot - 1:1000

Optimal dilutions should be determined by each laboratory for each application.

References

- 1. Jones, S.M. et al. (2003) J. Biol. Chem. 278:10257.
- 2. Dixon, R.A. et al. (1988) Proc. Natl. Acad. Sci. USA 85:416.
- 3. Luo, M. et al. (2005) J. Biol. Chem. 280:40609.
- 4. Funk, C. (2001) Science 294:1871.

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